Reference: *Biol. Bull.* **169**: 696–701. (December, 1985)

INHIBITION OF SEA URCHIN EGG MITOSIS BY RETINOIC ACID AFTER NEAR-UV LIGHT EXPOSURE

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ABSTRACT

Not only can chemicals enhance the cellular damage of near-UV light (photosensitization), but we now report that previous exposure to near-UV light can enhance chemically induced cellular damage. Fertilized sea urchin eggs were used to test all trans retinol, retinal, and retinoic acid as near-UV photosensitizers. Inhibition of cell division was not observed when fertilized eggs were exposed to the light in the presence of retinol or retinal. Retinoic acid and UV-exposure together partially prevented cell division. When fertilized or unfertilized eggs were pre-exposed to near-UV light and then 10^{-4} *M* retinoic acid added to the cultures, cell division was totally prevented. The inhibition of cell division was not observed due to any combination of UV exposure and/or addition of retinol or retinal. This work shows that near-UV light can sensitize the direct retinoic acid-mediated prevention of sea urchin egg division.

INTRODUCTION

Chemicals can enhance the cellular photodamage of near-UV light (photosensitization). We now report that near-UV light can enhance chemical damage to cells. Several previous reports (Epstein, 1977; Forbes *et al.*, 1979, 1980) have shown that after exposure of mice to non-toxic levels of UV-B radiation, the feeding of non-toxic levels of all-*trans*-retinoic acid (RA) altered the growth pattern of their skin cells. They developed tumors much more readily than the UV-irradiated or the RA-fed animals not exposed to this light.

We have been concerned that retinoids may be involved in the UV-photosensitization of ocular tissues via the formation of toxic photoproducts. We found, however, that this hypothesis was not upheld by experimentation, since neither retinol, retinal, nor retinoic acid, after they were exposed to long wavelength UV-light (365 nm), adversely influenced the survival or mitosis of sea urchin eggs. While yellow solutions of vitamin A become irreversibly bleached within a few hours by exposure to near-UV light (365 nm ± 20 nm; $50W/M^2$), the bleached products of vitamin A did not kill or stop the growth of dinoflagellates. Subsequently, experiments were undertaken using fertilized sea urchin eggs as test systems and all-*trans* retinol, retinal, and retinoic acids as potential photosensitizers. No photosensitization effects were observed. However, when eggs were exposed to near-UV light (as above) only the addition of the retinoid retinoic acid to the cultures prevented the division of sea urchin eggs.

Received 12 April 1985; accepted 20 August 1985.

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Materials and Methods

Eggs and sperm of sea urchins (*Arbacia punctulata* and *Lytechinus variegatus*) were obtained by injecting the animals with 0.5 ml of 3 *M* KCl. Millipore-filtered seawater was used for all collections and washings. Eggs were washed three times before use. Sperm samples were suspended in millipore-filtered seawater and diluted 20 fold before addition to egg suspensions for fertilization.

All-trans retinol, retinal, and retinoic acid (Sigma Chemical Co.) were dissolved in 100% methanol at 5 m*M*. Methanol solutions of the retinoids (0.1 ml) were added to seawater containing eggs (5 ml) to give a final concentration of 0.1 m*M*. The addition of methanol alone did not influence the fertilization or cell division processes of near-UV irradiated (as below) or unirradiated eggs.

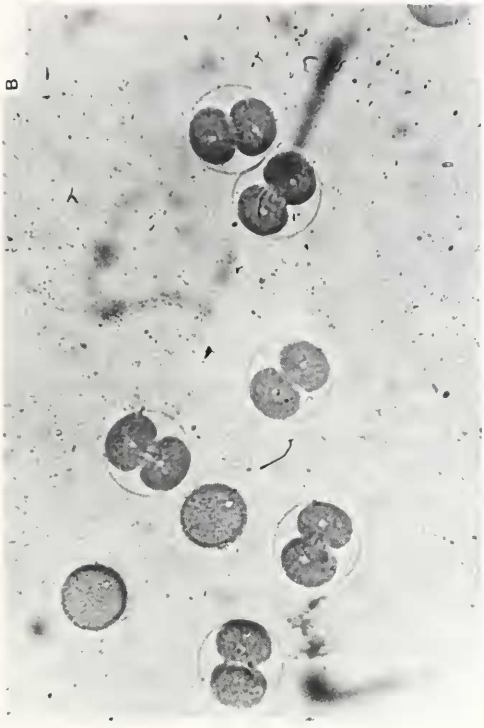
The lamp used to irradiate the eggs and the retinoic acid solutions was a long-wavelength emitting UV grid lamp 9PCQ 008L; Ultraviolet Products, Inc. long-wave UV meter (J 221) was $20W/M^2$ at a distance of 6 inches. The position of the egg suspension and the time of exposure of the eggs were varied from 2 to 24 h, providing a range of exposures between 1.4×10^5 and $1 \times 6\ 10^6$ J/M^2.

Experiments were carried out as in previous studies (Zigman and Hare, 1976; Zigman and Gilman, 1980). Eggs were obtained from several sea urchins. Five-ml portions of the stock suspensions were poured into 10-ml pyrex beakers and kept under the near-UV lamp, as above, with a 2-mm pyrex watch glass covering them. The suspensions were irradiated for 2, 3, 4, and 24 h in a water bath at 20°C. At the appropriate times, the eggs were removed from the UV chambers and sperm suspen-

Table I

Effects of retinoic acid (RA) and n-UV light on sea urchin egg development

Treatment	First Division		
	Time post-fertilization	% Dividing	One day embryonic development
1. None	60 min	90	Normal
2. Plus MeOH at 20 μl/ml	60 min	90	Normal
3. Plus RA at 10^{-4} *M*	70 min	80	Slightly delayed
4. Exposed to N-UV light through pyrex glass at $20W/M^2$ for 24 h	90 min	89	Slightly delayed
5. Plus RA (10^{-4} *M*) and exposed to N-UV as above for 1 h simultaneously	70 min	80	Slightly delayed
6. Same as 5. for 2 h	85 min	50	To 64–128 cells
7. Same as 5. for 3 h	None	None	None
8. Exposed to N-UV light (as above) for 24 h; than MeOH added-(20 μl/ml)	90 min	80	Slightly delayed
9. Plus RA (10^{-4} *M*) after N-UV exposure			
(as above) for: 2 h	65 min	80	Mostly normal
3 h	70 min	50	To 64 or 128 cells
4 h	90 min	2	None
24 h	None	None	None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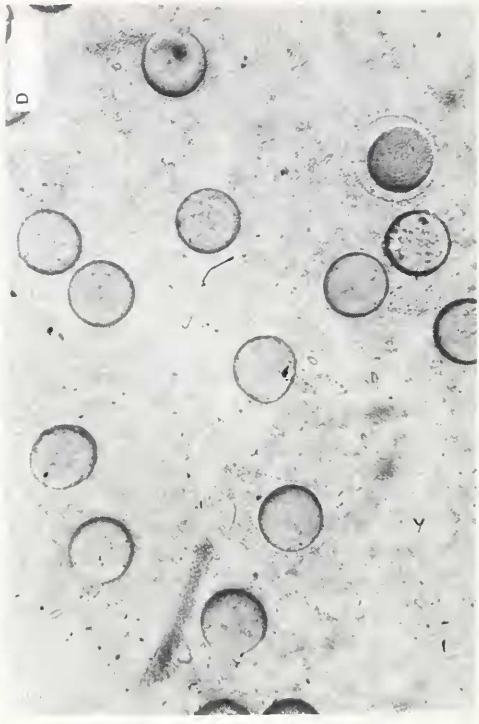
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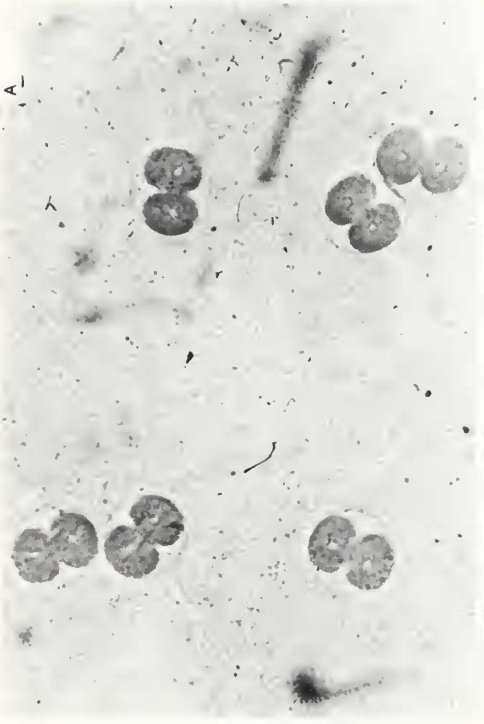
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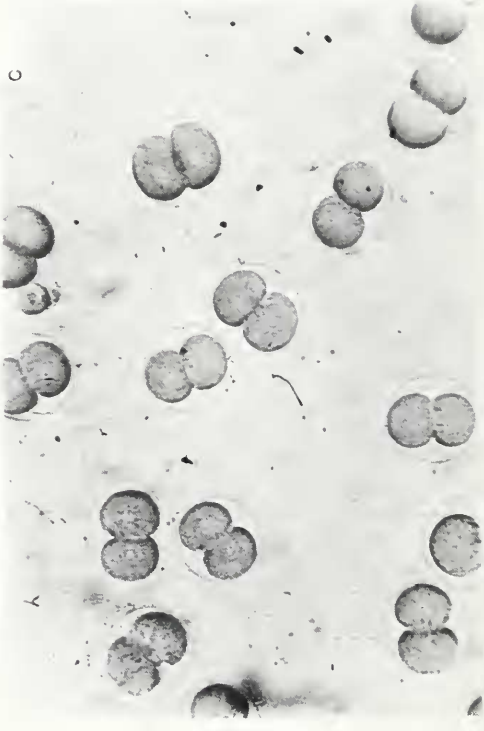
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sions were added to fertilize them. Fertilization membranes rose in 3 min, at which time the retinoid to be tested was added. A binocular compound microscope was used to observe the developmental events, and a Pentax Spotmatic 35 mm camera, attached through an adaptor, was used to photograph the eggs. In accompanying studies, eggs were fertilized and observed after UV exposure only, after retinoid exposure only, and after prior exposure of the eggs to retinoids and then to the near-UV light. None of the retinoids previously exposed to UV and added to sea urchin eggs influenced their division.

In another experiment, 2 μCi of 3H-thymidine (20 m Ci per mM) was added to the 5 ml of seawater in dishes containing freshly fertilized sea urchin eggs. At times from 20 to 100 minutes, 5 ml of 10% TCA was added to the dish to terminate incorporation and the cells were harvested and dissolved in 0.1 N NaOH. Aliquots of the precipitated material from each plate were counted in a Packard Tri-carb liquid scintillation counter. The categories studied were controls, UV only, 10^{-4} M retinoic acid only, and eggs UV-irradiated prior to the addition of retinoic acid.

Results

Table I summarizes the findings of our experiments. We found no photosensitizing action for UV-light exposure of any of the retinoids, and none of the retinoids were photo-oxidized to toxic products as tested against sea urchin egg mitotic activity. Methanol solutions of retinol had no influence on sea urchin mitosis whether in natural yellow form or bleached by near-UV light for four hours. Retinoic acid alone at 0.1 mM had little effect on sea urchin egg division or subsequent growth. When eggs were exposed to both retinoic acid and near-UV radiation together, cell division was markedly inhibited (see Table I). A dramatic total inhibition of mitosis also resulted when unfertilized sea urchin eggs were exposed to near-UV light for a period as short as 3 h, followed by addition of 0.1 M retinoic acid at 3 min postfertilization. Similar results were obtained when the retinoic acid was added to irradiated eggs before fertilization.

The near-UV enhanced prevention of sea urchin egg mitosis by retinoic acid is clearly shown in Figure 1, which shows the morphologic appearances of eggs treated as stated above. There is no influence of any of these treatments on the fertilization process as shown by the prompt appearance of the fertilization membranes. Only when the eggs were exposed to both factors or were pre-exposed to near-UV light and then retinoic acid was added to their suspensions (in this case after fertilization) was cell division prevented.

Figure 2 illustrates a time course of 3H-Tdr incorporation into the DNA of the developing sea urchin eggs. UV exposure of the eggs had little influence on Tdr incorporation. Retinoic acid alone inhibited Tdr incorporation after 40 min. Retinoic acid added to the system after UV-irradiation led to cessation of DNA synthesis. The degree of inhibition was much greater than for retinoic acid alone up to 40 min, but subsequently no further incorporation occurred.

The amount of inhibition of DNA synthesis due to the toxicity of retinoic acid alone was insufficient to totally stop mitosis, whereas the same concentration of retinoic acid added to the eggs after pre-UV irradiation totally prevented mitosis.

FIGURE 1. Sea urchin (*Lytechinus variegatis*) eggs at one hour postfertilization with the following treatments: (A) control; (B) with 10^{-4} M RA added; (C) after exposure to near-UV light for four hours before fertilization; (D) with 10^{-4} M RA added after pre-exposure to near-UV light for four hours.

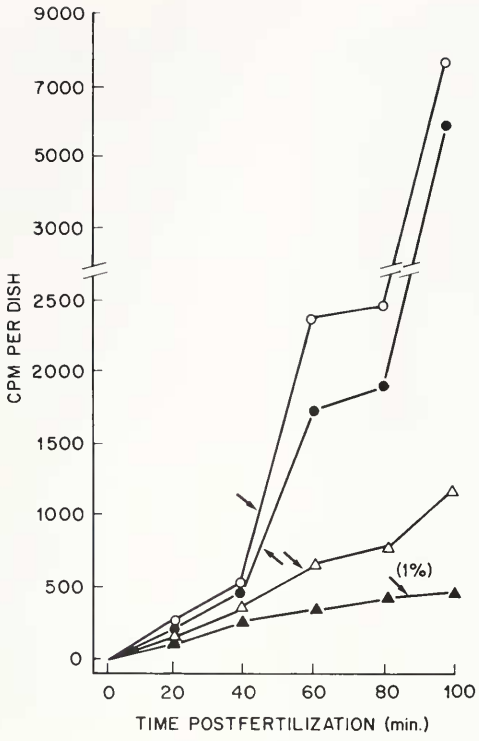

FIGURE 2. Time course of incorporation of ^{3}H-Tdr into the DNA of fertilized sea urchin eggs. ↗ = first division occurs, 1% = percent of cells dividing; open circles = control; closed circles = irradiated with no additives; open triangles = retinoic acid added, but no irradiation; closed triangles = 8 h UV pre-irradiation, then retinoic acid added. Conditions of the experiment are stated in the Materials and Methods section.

DISCUSSION

This study demonstrates that near-UV light at irradiances not greater than those found in the natural environment or derived from many artificial lamps, can sensitize cells so that non-toxic biochemicals become toxic to them. Because mitosis is a complicated process, it is not possible to suggest a specific mechanism of action for the near-UV chemosensitization of sea urchin eggs by retinoic acid. Further studies are needed.

Whatever the mechanism is, it appears to be related to an early inhibition of DNA synthesis which interferes with cell division and further development. While retinoic acid alone adversely influences DNA synthesis, the effect occurs too late or at too minor a degree to stop mitosis. On the other hand, pre-UV-exposed eggs do suffer sufficient DNA synthesis inhibition to stop mitosis.

The enhancement of skin tumor development due to pre-exposure to near-UV radiation and then retinoic acid application was shown by others (Epstein, 1977; Forbes *et al.*, 1979) to enhance abnormal development of cells. Thus, we report a phenomenon not generally known or understood, but which seems to have a great significance in the study of near-UV light effects on many types of cells studied in biology and medicine.

ACKNOWLEDGMENT

Support: National Eye Institute (NIH) EY 00459; and Research to Prevent Blindness, Inc.

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